Reprinted from Journal of the American Mosquito Control Association
Vol 6 September 1990 Number 3

ATTRACTION OF MOSQUITOES TO DIETHYL METHYLBENZAMIDE AND ETHYL HEXANEDIOL[1]

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ABSTRACT. Studies by prior workers have shown that insect repellents can act as attractants when present as low concentrations, deposits or residues. In the present study deet and ethyl hexanediol were tested in 2-fold serial doses from 1.9×10^{-9} to 1.6×10^{-2} mg/cm² on the forearms of volunteers against colonized *Anopheles albimanus*, *Aedes aegypti* and *Ae. taeniorhynchus*. Both compounds were significantly repellent at the high end of the dose range, as expected. Neither was significantly attractant to *An. albimanus* in low doses. However, deet was significantly attractant to *Ae. aegypti* in the dose range 7.6×10^{-8} to 1.2×10^{-4} mg/cm² and to *Ae. taeniorhynchus* in the dose ranges 1.9×10^{-9} to 3.1×10^{-8} mg/cm² and 2.0×10^{-6} to 2.5×10^{-4} mg/cm². Ethyl hexanediol was significantly attractant to *Ae. taeniorhynchus* in the dose range 1.9×10^{-9} to 6.2×10^{-5} mg/cm². Based on these results and prior work of V. G. Dethier and C. N. E. Ruscoe, a model sequence of the effects of chemicals on insects with increasing dose was developed. It was concluded that the labels of commercial repellents should be amended to include instructions to wash off or reapply the repellent when it is no longer effective.

INTRODUCTION

Several investigators have reported that repellents can act as attractants when present as low concentrations, deposits or residues. In the laboratory Hocking (1961) found that vapors of butoxy polypropylene glycol (butoxypropanediol polymer) were attractant to *Aedes aegypti* (Linn.) in a T-tube olfactometer. Kost et al. (1971) reported that vapors of deet (*N,N*-diethyl-3-methylbenzamide) and benzimine (*N*-benzoylhexamethylenimine) were attractant to *Ae. aegypti* at low concentrations, and Potapov et al. (1977) reported similar effects for rebemid (*N,N*-diethylbenzamide) and repellent P-633 (cyclopentanone-2-carboxylic acid) against *Ae. aegypti*.

In the field Dubitskii (1966) found that vapors of dimethyl phthalate, repudin (composition not given), benzimine and deet were attractant to *Anopheles hyrcanus* (Pallas), *Aedes cinereus* Meigen, *Aedes vexans* (Meigen), *Aedes caspius* (Pallas) and *Culex modestus* Ficalbi. Potopov et al. (1977) found that rebemid on clothing and 1% deet on the skin were attractant to *Coquillettidia richiardii* (Ficalbi) and concluded that repellents should be washed off the skin when their repellent action ceases.

In an earlier study we observed attractancy at low doses and repellency at high doses in laboratory trials of 2 cyclic analogs of lactic acid, methyl-6-pentyl-1-cyclohexene-1-carboxylate and 4-butyl-2,3-morpholinedione, against *Ae. aegypti* (Skinner et al. 1980). The present study was conducted to determine the attractancy or repellency of deet and ethyl hexanediol (2-ethyl-1,3-hexanediol) for *Anopheles albimanus* Wied., *Ae. aegypti*, and *Aedes taeniorhynchus* (Wied.) over a wide range of doses. A preliminary report was given by Mehr and Rutledge (1985).

MATERIALS and METHODS

Test insects: Three laboratory colonies of mosquitoes were used in the study: *An. albimanus* and *Ae. taeniorhynchus* obtained from Carl E. Schreck, Insects Affecting Man and Animals Research Laboratory, U.S. Department of Agriculture, Gainesville, Florida, and *Ae. aegypti* obtained from Abdul A. Khan, University of California at San Francisco. Larvae were reared at 27 °C on a diet of floating catfish food (Continental Grain, Chicago, IL). Adults were maintained under a 12:12 h photoperiod at 27°C and 80% RH on white rabbits and 10% sucrose solution. Tests were conducted with 5- to 15-day-old nulliparous females.

Test materials: Test materials were technical grade deet (McLaughlin Gormley King Company, Minneapolis, MN) and technical grade ethyl hexanediol (Eastman Organic Chemicals, Rochester, NY). Materials were tested in 2-fold serial dilutions in ethanol. Dilutions were calculated to provide doses of 1.9×10^{-9} to $1.6 \times$

[1] The opinions and assertions contained herein are the private views of the authors and should not be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. Use of a trade name does not indicate official endorsement or approval of the use of the product. Human subjects participating in this study gave free and informed voluntary consent, and the investigators adhered to Army Regulation 70-25 and U.S. Army Medical Research and Development Command Regulation 70-25 on the use of volunteers in research.

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10^{-2} mg/cm^2 of active ingredient when 0.025 ml of solution was applied to a 6.6 cm^2 test area.

Test subjects: Five volunteers (4 male and 1 female) and 6 alternates (5 male and 1 female) participated in the study.

Test methods: A method of the American Society for Testing and Materials (1983) was adapted for use in the study. Five 2.9-cm diameter (6.6 cm^2) circular test areas were imprinted on the flexor region of the forearm of a volunteer with a stamp and inking pad. The 5 test areas were treated at random with 0.025 ml of 4 serial dilutions of the test repellent and a control (ethanol) using a micropipet and a glass spreading rod. After 5 min a 4 × 5 × 18 cm clear acrylic plastic test cage containing 15 mosquitoes was bound to the forearm with 1-inch Velcro tape, and a slide was withdrawn to expose the 5 test areas through matching holes in the floor of the cage. The number of mosquitoes biting in each of the 5 test areas was recorded at the end of 90 sec. The mosquitoes were then narcotized with a jet of carbon dioxide, the slide was replaced and the cage was removed.

New mosquitoes were used in each 90-sec trial. The initial range of doses applied (2.0×10^{-3}, 4.0×10^{-3}, 8.0×10^{-3} and 1.6×10^{-2} mg/cm^2) was progressively extended to lower and lower levels in successive trials. A minimum of 8 replications on at least 2 volunteers was performed on each range of doses tested.

Data analysis: The percent attractancy or repellency of the test material for a given species at a given dose was calculated as:

Percent attractancy/repellency

$$= 100 - \frac{\text{total no. bites on treatment}}{\text{total no. bites on control}} \times 100$$

This value expresses percent attractancy/repellency in terms of the concurrent control to adjust for variation due to differences among test subjects and the date and time of testing. It is negative when more bites occur on the treatment than on the control (attractancy) and positive when more bites occur on the control than on the treatment (repellency).

Since deet and ethyl hexanediol were known to be repellent to *An. albimanus*, *Ae. aegypti* and *Ae. taeniorhynchus* at the highest doses used (Rutledge et al. 1983), the total range of doses tested (1.9×10^{-9} to 1.6×10^{-2} mg/cm^2) was divided into a dilute range and a repellent range for analysis. To be conservative, the dilute range was defined to include all doses up to and including the dose next higher than the highest observed attractant dose (Figs. 1–3). The repellent range was defined to include all higher doses. By these rules, the observed values of percent attractancy/repellency in the dilute range were either negative (attractancy) or positive (repellency); observed values of percent attractancy/repellency in the repellent range were always positive (repellency).

Three statistical tests were performed on the values of percent attractancy/repellency observed within the dilute range: 1) The *t* test (Steel and Torrie 1980) was performed to determine if the mean percent attractancy/repellency within the dilute range was significantly less than zero (i.e., negative), indicating significant attraction. 2) The numbers of attractant (negative) and repellent (positive) values observed within the dilute range were compared with tables of the binomial distribution (Beyer 1968) to determine if the occurrence of attractant values was significantly more frequent than the occurrence of repellent values. 3) The runs test (Beyer 1968) was performed to determine if the sequence of attractant and repellent values was random within the dilute range or if the attractant (negative) and repellent (positive) values were significantly clustered. The 5% error rate was employed in all tests of significance.

RESULTS

An. albimanus: A total of 2,895 bites were recorded in 278 single-cage trials of deet against *An. albimanus* for an overall mean of 10.4 bites per trial. The dilute range was 1.9×10^{-9} to 2.0×10^{-3} mg/cm^2 (Fig. 1). Neither the *t* test, binomial probability nor runs test was statistically significant (Tables 1–3).

A total of 1,745 bites were recorded in 144 single-cage trials of ethyl hexanediol against *An. albimanus* for an overall mean of 12.1 bites per trial. The dilute range was 1.9×10^{-9} to 2.0×10^{-3} mg/cm^2 (Fig. 1). Neither the *t* test, binomial probability, nor runs test was statistically significant (Tables 1–3).

Ae. aegypti: A total of 4,307 bites were recorded in 297 single-cage trials of deet against *Ae. aegypti* for an overall mean of 14.5 bites per trial. The dilute range was 7.6×10^{-9} to 2.5×10^{-4} mg/cm^2 (Fig. 2). (The 2 lowest doses of the standard range, 1.9×10^{-9} and 3.8×10^{-9} mg/cm^2, were not tested in this case.) Both the *t* test and the binomial probability were statistically significant (Tables 1 and 2). The mean percent attractancy/repellency within the dilute range was −5.6% (Table 1). The largest negative (attractant) value observed was −33% at 3.1×10^{-5} mg/cm^2 (Fig. 2). Twelve of the 16 attractancy/repellency values observed in the dilute range were negative (attractant) (Table 2). The runs test was not statistically significant (Table 3).

A total of 5,747 bites were recorded in 406 single-cage trials of ethyl hexanediol against *Ae. aegypti* for an overall mean of 14.2 bites per

trial. The dilute range was 1.9×10^{-9} to 2.0×10^{-3} mg/cm^2 (Fig. 2). Neither the *t* test, binomial probability nor runs test was statistically significant (Tables 1–3).

Ae. taeniorhynchus: A total of 2,011 bites were recorded in 208 single-cage trials of deet against *Ae. taeniorhynchus* for an overall mean of 9.7 bites per trial. The dilute range was 1.9×10^{-9} to 5.0×10^{-4} mg/cm^2 (Fig. 3). Both the *t* test and the runs test were statistically significant (Tables 1 and 3). The mean percent attractancy/repellency within the dilute range was −12.5% (Table 1). The largest negative (attractant) val-

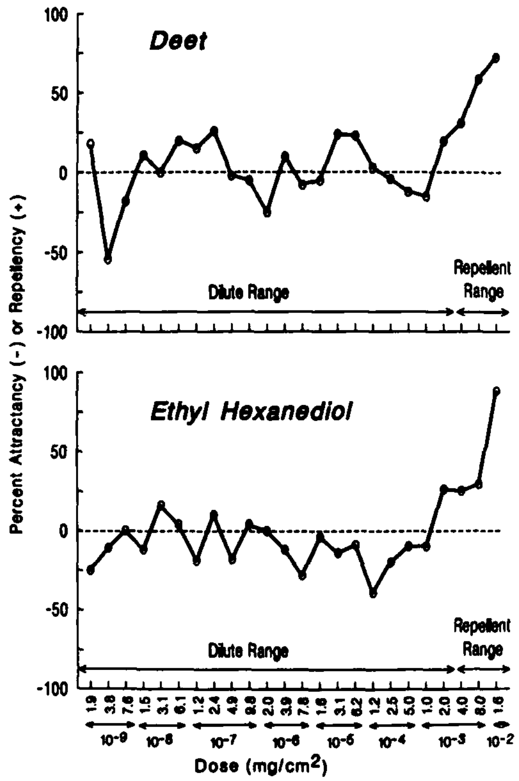

Fig. 1. Percent repellency/attractancy of serial doses of deet and ethyl hexanediol in tests against *Anopheles albimanus*.

Table 2. Data for the binomial probability test of frequencies of attractant and repellent observations (Figs. 1–3) in tests of deet and ethyl hexanediol in the dilute dose-range. *P* is the exact probability of observing an equal or larger number of attractant values if $\theta = 0.5$ (Beyer 1968).

	Number of observations		
	Attractant	Repellent	*P*
An. albimanus			
Deet	11	10	0.50
Ethyl hexanediol	14	7	0.09
Ae. aegypti			
Deet	12	4	0.04
Ethyl hexanediol	7	14	0.96
Ae. taeniorhynchus			
Deet	13	6	0.08
Ethyl hexanediol	11	6	0.17

Table 3. Data for the runs tests of the sequence of attractant and repellent values (Figs. 1–3) observed for deet and ethyl hexanediol in the dilute dose-range. *P* is the exact probability of occurrence of an equal or smaller number of runs (Beyer 1968).

	Number of runs		
	Observed	Expected	*P*
An. albimanus			
Deet	11	11.5	0.50
Ethyl hexanediol	11	10.3	0.72
Ae. aegypti			
Deet	6	7.0	0.34
Ethyl hexanediol	11	10.3	0.72
Ae. taeniorhynchus			
Deet	4	9.2	0.01
Ethyl hexanediol	10	8.8	0.82

Table 1. Data for the *t* test of observed values (Figs. 1–3) for attractancy and repellency of deet and ethyl hexanediol in the dilute range. *P* is the probability of a smaller value of *t* (sign considered) if $\mu \geq 0$ (Fisher and Yates 1963).

	Number of observations	Mean	Standard error	*t*	*P*
An. albimanus					
Deet	21	−1.000	4.274	−0.234	0.44
Ethyl hexanediol	21	−5.476	3.569	−1.534	0.07
Ae. aegypti					
Deet	16	−5.562	3.036	−1.832	0.04
Ethyl hexanediol	21	+3.762	2.131	+1.765	0.95
Ae. taeniorhynchus					
Deet	19	−12.474	5.445	−2.291	0.02
Ethyl hexanediol	17	−15.706	7.223	−2.174	0.02

ues observed were −45% at 1.5×10^{-8} mg/cm^2 and −67% at 1.2×10^{-4} mg/cm^2 (Fig. 3). The longest runs of negative (attractant) values observed were a run of 5 consecutive negative values from 1.9×10^{-9} to 3.1×10^{-8} mg/cm^2 and a run of 8 consecutive negative values from 2.0×10^{-6} to 2.5×10^{-4} mg/cm^2 (Fig. 3). The binomial probability was not statistically significant (Table 2).

A total of 2,344 bites were recorded in 231 single-cage trials of ethyl hexanediol against *Ae. taeniorhynchus* for an overall mean of 10.1 bites per trial. The dilute range was 1.9×10^{-9} to 1.2×10^{-4} mg/cm^2 (Fig. 3). The t test was statistically significant (Table 1). The mean percent attractancy/repellency within the dilute range was −15.7%. The largest negative (attractant) value observed was −95% at 7.6×10^{-9} mg/cm^2 (Fig. 3). Neither the binomial probability nor the runs test was statistically significant (Tables 2 and 3).

DISCUSSION

The smallest dose of deet used in the study was 1.9×10^{-9} mg/cm^2 (Figs. 1–3). Converting

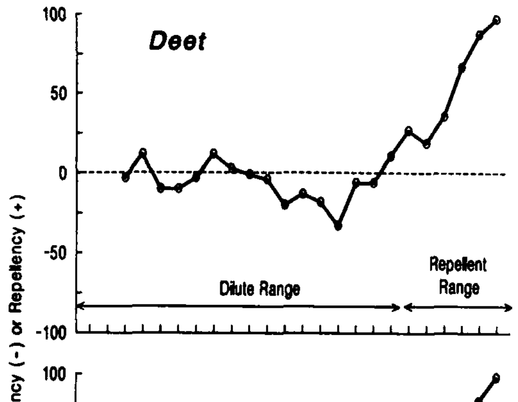

Fig. 2. Percent repellency/attractancy of serial doses of deet and ethyl hexanediol in tests against *Aedes aegypti*.

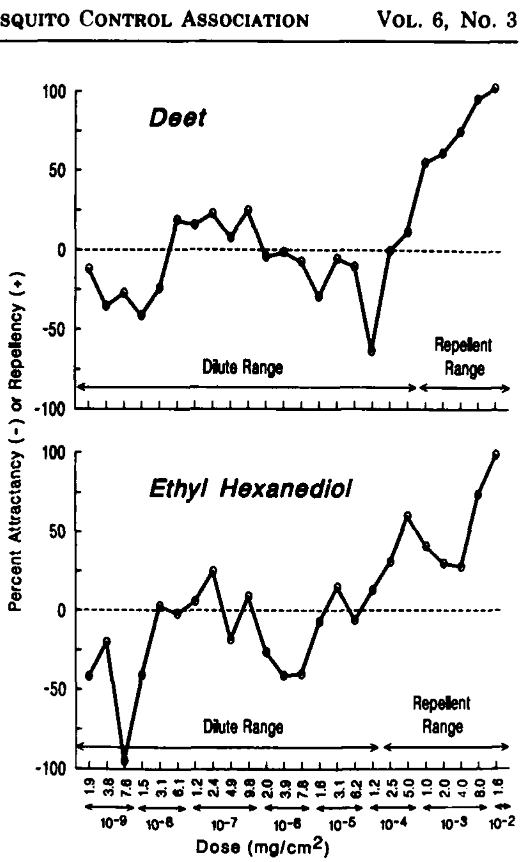

Fig. 3. Percent repellency/attractancy of serial doses of deet and ethyl hexanediol in tests against *Aedes taeniorhynchus*.

1.9×10^{-9} mg/cm^2 to g/cm^2 and dividing by the molecular weight of deet (191.3) gives 9.9×10^{-15} moles/cm^2. Multiplying this figure by Avogadro's number (6.23×10^{23}) gives 6.2×10^{9} molecules/cm^2 of skin. The analogous calculation for ethyl hexanediol gives 8.1×10^{9} molecules/cm^2 of skin. These figures distinguish our study sharply from that of Davenas et al. (1988) in which anti-immunoglobulin E was reported to induce the release of histamine by human polymorphonuclear basophils at dilutions up to 1×10^{120}. At such high dilutions there is virtually no possibility that any anti-immunoglobulin E remained in the reagent fluid (Maddox et al. 1988).

In the present study low doses of deet were attractant to *Ae. aegypti* and *Ae. taeniorhynchus* but not to *An. albimanus* (Tables 1–3). Since completion of the study the same effect has been observed in laboratory trials of controlled-release formulations of deet against both *Ae. aegypti* and *Ae. taeniorhynchus* (Gupta and Rutledge 1989). These results confirm the report of Kost et al. (1971) that low concentrations of deet are attractant to *Ae. aegypti* and call into question the report of Potapov et al. (1977) that 1% deet is repellent to *Ae. aegypti* for up to 7

days after application to the forearm. We have found no prior report of the attraction of *Ae. taeniorhynchus* to deet. Attraction of *Anopheles farauti* Laveran, *Aedes kochi* (Doenitz), *Aedes carmenti* Edwards, and *Culex annulirostris* Skuse to residues of deet and permethrin in the field was reported earlier (Gupta et al. 1987).

In the present study low doses of ethyl hexanediol were attractant to *Ae. taeniorhynchus* but not to *An. albimanus* or *Ae. aegypti* (Tables 1-3). We have found no prior report of the attraction of mosquitoes to ethyl hexanediol.

Prior workers have reported that low concentrations of deet and other repellents were attractant to other Diptera. Deet was attractant to the sand fly *Phlebotomus papatasi* Scopoli (Psychodidae) (Sabitov 1985). Deet, dimethyl phthalate, benzimine, and repudin were attractant to the biting midges *Culicoides pulicaris* Linn. and *Culicoides puncticollis* Becker (Ceratopogonidae) (Dubitskii 1966). Sulfobenzamide repellents were attractant to the black flies *Gnus cholodkovskiy* (Rubzov) and *Simulium galeratum* Edwards (Simuliidae) and the horse flies *Hybomitra bimaculata* Macquart and *Hybomitra distinguenda* Verrall (Tabanidae) (Potapov et al. 1977). Diallyl phthalate repellents were attractant to the olive fruit fly *Dacus oleae* Gmelin (Diptera: Tephritidae) (Wright 1982). Butoxy polypropylene glycol was attractant to the house fly *Musca domestica* Linn. (Diptera: Muscidae) (Hocking 1961). MGK Repellent 11 (1,5a,6,9,9a,9b-hexahydro-4a(4H)-dibenzofurancarboxyaldehyde) was attractant to the stable fly *Stomoxys calcitrans* (Linn.) (Muscidae) (Yeoman and Warren 1970).

On the other hand many materials that are normally thought of as attractants have been reported to be repellent at high concentrations.[5] Smith et al. (1970) reported that lactic acid was attractant to *Ae. aegypti* at concentrations normally present on the skin and in the breath but repellent at a higher (3.6 mg/cm^2) concentration.[6] Kramer et al. (1980) reported that butyric acid was an oviposition attractant for *Culiseta incidens* (Thomson) at low concentrations and an oviposition repellent at high concentrations.

Although carbon dioxide is a potent attractant for mosquitoes in nature (Reeves 1953), its effects in laboratory experiments have been variable (Gillies 1980). Willis (1947) and Willis and Roth (1952) demonstrated that it can be attractant, inert or repellent, depending on the kind of olfactometer used. Their data for female *Ae. aegypti* in a small-cage olfactometer (Table 1 of Willis and Roth 1952) indicate that the repellent effect increases with increasing concentrations (0.1–50%) of carbon dioxide.

Nakagawa et al. (1971) reported that high concentrations of trimedlure were repellent to the Mediteranean fruit fly *Ceratitis capita* (Wied.) (Tephritidae). Barrows (1907) reported that 8% ethyl acetate was attractant to the vinegar fly *Drosophila ampelophila* Loew (Diptera: Drosophilidae) at a distance, but repellent at close range. In this case, it would seem that the gradient in space produced by diffusion and convection through increasing distance would be equivalent to the graded doses in the present study. Reed (1938) reported that solutions of acetic acid above 5% and of ethanol above 25% were repellent to *Drosophila melanogaster* Meigen. Triethylamine hydrochloride was repellent to *Hippelates collusor* (Townsend) (Chloropidae) (Mulla et al. 1976). Aliphatic aldehydes were repellent to the black blow fly *Phormia regina* Meigen (Calliphoridae) (Dethier 1954a). Secondary amyl mercaptan was repellent to *Lucilia sericata* (Meigen) (Calliphoridae) (Hoskins and Craig 1934). Ammonia, ethanol and isovaleraldehyde were repellent to the house fly (Wieting and Hoskins 1939, Dethier et al. 1952, Dethier 1954a).

Dethier (1954a) published a figure showing the succession of subliminal, attractant and repellent effects of increasing concentrations of isovaleraldehyde on the house fly.[7] This sequence can be represented by the series

Neutral → Attractant → Neutral → Repellent

in which the term "Neutral" includes both the subliminal and the transitional effects. In the present study this sequence of effects was observed in tests of deet against *Ae. aegypti* (Fig.

[5] A related topic is the case in which a material is attractant to one species but repellent to another. Thus, geraniol (3,7-dimethyl-2,6-octadien-1-ol), a well-known commercial attractant for the Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), was repellent to *Ae. aegypti* in an *in vitro* blood-feeding test system: $ED_{50} = 0.031$ mg/cm^2 (95% CL = 0.000, 0.060); slope = −1.614 (SE = 0.163) (unpublished data, Letterman Army Institute of Research).

[6] Kostin (1984) reported that lactic acid was repellent to *Ae. aegypti* at low atmospheric pressure but attractant at high atmospheric pressure. It is not clear how this observation relates to the present discussion. According to the law of partial pressures (Dalton's law) the rate of evaporation of lactic acid from the skin, and its consequent concentration in the air above the skin, is not affected by atmospheric pressure.

[7] Dethier reprinted this same figure in his review of the physiology of olfaction in insects (Dethier 1954b), stating that "For every chemically pure attractant thus far tested, there can be found a concentration at which it becomes repellent." He did not mention the converse, that repellents may become attractant at low concentrations.

2) and of ethyl hexanediol against *Ae. taeniorhynchus* (Fig. 3).

However, no statistically significant attractant dose was observed in tests of deet and ethyl hexanediol against *An. albimanus* (Fig. 1) or in tests of ethyl hexanediol against *Ae. aegypti* (Fig. 2). Moreover, the runs test (Table 3) identified 2 distinct attractant dose ranges (1.9×10^{-9} to 3.1×10^{-8} mg/cm^2 and 2.0×10^{-6} to 2.5×10^{-4} mg/cm^2) in the case of deet against *Ae. taeniorhynchus* (Fig. 3). These variations of Dethier's sequence can be represented by the series

$$(\text{Neutral} \rightarrow \text{Attractant})_N \rightarrow \text{Neutral} \rightarrow \text{Repellent}$$

in which N can be 0, 1 or 2.

Potapov et al. (1977) reported that 40% deet applied to the forearm was alternately repellent and attractant to *Ae. communis* for 3 days after application.[8] The results obtained in tests of deet against *Ae. taeniorhynchus* in the present study (Table 3 and Fig. 3) could also be interpreted in terms of alternating repellent and attractant effects. However, we have interpreted the data in terms of alternating neutral and attractant effects because the repellent values were not statistically significant within the dilute range.

A number of thiocyanate, organophosphate, chlorinated hydrocarbon and pyrethroid insecticides, including permethrin, have been shown to be repellent to mosquitoes at sublethal doses, and a number of repellents, including deet and ethyl hexanediol, have been shown to be toxic to mosquitoes at high doses (Rutledge et al. 1981). Potapov and Bogdanova (1974) reported that 10–30% solutions of repellents R-2 (benzoic acid diethylamide), R-31 (caproic acid diethylamide), R-228 (m-toluic acid *N*-piperidylamide), R-320 (furan-2-carboxylic acid diethylamide) and R-386 (α-chloropropionic acid diethylamide) were more repellent than 40–50% solutions because of narcotic and toxic effects at the higher concentrations.

Ruscoe (1977) published a figure showing the succession of toxic, repellent/antifeedant and subliminal effects of progressively older residues of permethrin on the diamondback moth *Plutella xylostella* (Linn.) (Lepidoptera: Plutellidae). In this case it would seem that the gradient in time produced by the decay and dissipation of the residues would be equivalent to the graded doses in the present study. Accordingly, Ruscoe's sequence can be incorporated into that of Dethier by the series

$$(\text{Neutral} \rightarrow \text{Attractant})_N \rightarrow \text{Neutral} \rightarrow \text{Repellent} \rightarrow \text{Toxic}$$

in which the term "Repellent" includes both repellent and antifeedant effects.

Rani and Osmani (1984) have confirmed this model sequence of effects in tests of methoxychlor and cyphenothrin against the house fly. Even so, the model requires further research for full verification and should be regarded as tentative. For example, Riha et al. (1986) reported that the toxic effects of 0.05% permethrin applied to horses as a spray lasted longer than the repellent effects. This is contrary to expectation from the model sequence and contrary to the observed sequence for permethrin-treated cotton and cotton/nylon fabrics (Gupta et al. 1989).

[8] Potapov et al. (1977) interpreted this and related experiments (Potapov and Vladimirova 1970, Vladimirova, 1969, 1970a, 1970b) primarily in terms of changes in ambient meterological conditions and the physiological state of the test insects during the test.

CONCLUSIONS

According to Sabitov (1985) instructions for use of repellents in the Soviet Union include information on the attractant effects of repellent residues on the skin. From results of the present study, we conclude that the U.S. Environmental Protection Agency should require this information in the "Directions for Use" section of repellent labels, along with instructions to wash off or reapply the repellent when it is no longer effective. In this connection it may be noted that the Agency has recently issued a Consumer Bulletin providing use precautions for deet based on adverse reaction reports involving children (Anonymous 1989). The precautions recommended by the Agency include: "Do not reapply or saturate. Wash treated skin with soap and water after and between uses."

ACKNOWLEDGMENTS

The volunteers and alternates received 19,049 bites in the course of the study. We thank J. L. Inase, V. E. Meixsell, J. J. Newland, P. I. Plamp, H. G. Semey, E. A. Shipwash and R. A. Wirtz for participation.

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